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(71) Applicant *(for all designated States except US)*: ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: AUDIA, James, E. [US/US]; 6449 Lakeside Woods Circle, Indianapolis, IN 46278 (US).

(74) Agents: GAYLO, Paul, J. et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).

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(54) Title: METHODS OF TREATING β-AMYLOID-ASSOCIATED CONDITIONS

(57) Abstract

This invention provides a method for treating a physiological disorder associated with β-amyloid peptide in a mammal which comprises administering to a mammal in need thereof an effective amount of a composition having serotonin reuptake inhibition activity.

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Title

METHODS OF TREATING
β-AMYLOID - ASSOCIATED CONDITIONS

Priority Claim

This application claims the benefit of U.S. Provisional Application No. 60/004178, filed September 22, 1995.

Background of the Invention

Since the discovery of serotonin (5-hydroxytryptamine, 5-HT) over four decades ago, the cumulative results of many diverse studies have indicated that serotonin plays a significant role in the functioning of the mammalian body, both in the central nervous system and in peripheral systems as well. Morphological studies of the central nervous system have shown that serotonergic neurons, which originate in the brain stem, form a very diffuse system that projects to most areas of the brain and spinal cord. R.A. O'Brien, Serotonin in Mental Abnormalities, 1:41 (1978); H.W.M. Steinbusch, HANDBOOK OF CHEMICAL NEUROANATOMY, Volume 3, Part II, 68 (1984); N.E. Anden, et al., Acta Physiologica Scandinavia, 67:313 (1966). These studies have been complemented by biochemical evidence that indicates large concentrations of 5-HT exist in the brain and spinal cord. H.W.M. Steinbusch, supra.

With such a diffuse system, it is not surprising that 5-HT has been implicated as being involved in the expression of a number of behaviors, physiological responses, and diseases which originate in the central nervous system. These include such diverse areas as sleeping, eating, perceiving pain, controlling body temperature, controlling blood pressure, depression, schizophrenia, and other bodily states. R.W. Fuller, BIOLOGY OF SEROTONERGIC TRANSMISSION, 221 (1982); D.J. Boullin, SEROTONIN IN MENTAL ABNORMALITIES 1:316 (1978); J. Barchas, et al., Serotonin and Behavior,(1973).

Serotonin plays an important role in peripheral systems as well. For example, approximately 90% of the body's serotonin is

synthesized in the gastrointestinal system, and serotonin has been found to mediate a variety of contractile, secretory, and electrophysiologic effects in this system. Serotonin may be taken up by the platelets and, upon platelet aggregation, be released such that the cardiovascular system provides another example of a peripheral network that is very sensitive to serotonin. Given the broad distribution of serotonin within the body, it is understandable that tremendous interest in drugs that affect serotonergic systems exists. In particular, serotonergic systems are of interest for the treatment of a wide range of disorders, including anxiety, depression, hypertension, migraine, compulsive disorders, schizophhrenia, autism, Parkinsonism, and Huntington's chorea, and cancer chemotherapy-induced vomiting. M.D. Gershon, et al., THE PERIPHERAL ACTIONS OF 5-HYDROXYTRYPTAMINE, 246 (1989); P.R. Saxena, et al., Journal of Cardiovascular Pharmacology, 15:Supplement 7 (1990).

Alzheimer's disease is a degenerative disorder of the human brain. Clinically, it appears as a progressive dementia. Its histopathology is characterized by degeneration of neurons, gliosis, and the abnormal deposition of proteins in the brain. Proteinaceous deposits (called "amyloid") appear as neurofibrillary tangles, amyloid plaque cores, and amyloid of the congophilic angiopathy. [For reviews, see, Alzheimer's Disease, (B. Reisberg, ed., The Free Press 1983).]

While there is no general agreement as to the chemical nature of neurofibrillary tangles, the major constituent of both the amyloid plaque cores and the amyloid of the congophilic angiopathy has been shown to be a 4500 Dalton protein originally termed β-protein or amyloid A4. Throughout this document this protein is referred to as β-amyloid peptide or protein.

β-amyloid peptide is proteolytically derived from a transmembrane protein, the amyloid precursor protein (APP). Different splice forms of the amyloid precursor protein are encoded by a widely expressed gene. see, e.g., K. Beyreuther and B. Müller-Hill, Annual Reviews in Biochemistry, 58:287-307 (1989). β-Amyloid peptide consists, in its longest forms, of 42 or 43 amino acid residues. J. Kang, et al., Nature (London), 325:733-736 (1987). These peptides, however, vary as to

their amino-termini. C. Hilbich, et al., Journal of Molecular Biology, 218:149-163 (1991).

Because senile plaques are invariably surrounded by dystrophic neurites, it was proposed early that β-amyloid peptide is involved in the loss of neuronal cells that occurs in Alzheimer's disease. B. Yankner and co-workers were the first to demonstrate that synthetic β-amyloid peptide could be neurotoxic in vitro and in vivo. B.A. Yankner, et al., Science, 245:417 (1989); See, also, N.W. Kowall, et al., Proceedings of the National Academy of Sciences, U.S.A., 88:7247 (1991). Other research groups, however, were unable to consistently demonstrate direct toxicity with β-amyloid peptide. See, e.g., Neurobiology of Aging, 13:535 (K. Kosik and P. Coleman, eds. 1992). Even groups receiving β-amyloid peptide from a common source demonstrate conflicting results. D. Price, et al., Neurobiology of Aging, 13:623-625 (1991)(and the references cited therein).

As mentioned supra, cells have alternative mechanisms for processing amyloid precursor protein which can result in the formation of the β -amyloid protein and subsequently, the senile plaques.

Because of the debilitating effects of Alzheimer's disease there continues to exist a need for effective treatments. This invention provides methods for the treatment of Alzheimer's disease and other conditions associated with β-amyloid peptide in mammals. Specifically, this invention provides methods of using selective serotonin reuptake inhibitors as a treatment for Alzheimer's disease and these other conditions.

Summary of the Invention

This invention provides a method for treating a physiological disorder associated with β-amyloid peptide in a mammal which comprises administering to a mammal in need thereof an effective amount of a composition having serotonin reuptake inhibition activity.

Detailed Description and Preferred Embodiments

β-amyloid peptide naturally occurs as a series of peptides which are 39 to 43 amino acids long, with the shorter, more soluble forms being present in cerebrovascular deposits and the longer forms being found primarily in senile plaques. F. Prelli, et al., Journal of Neurochemistry, 51:648-651 (1988). The primary structure of the 43 amino acid long peptide (β1-43) is depicted in SEQ ID NO:1:

```
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln      15
Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala      30
Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr              43
```

Even though the full length peptide of SEQ ID NO:1: has sufficient solubility in water for the following experiments, for the purposes of convenience, a more water-soluble form of the peptide is often desired. For that reason, the following examples were performed using peptides containing just the first 40 amino acids of the β-amyloid peptide (β1-40). The sequence of this preferred peptide is SEQ ID NO:2:

```
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln      15
Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala      30
Ile Ile Gly Leu Met Val Gly Gly Val Val                          40
```

It is understood by those in the art that other fragments of β-amyloid peptide, comprising amino-truncated, carboxy-truncated, or internal deletions, or any combination of these, as well as conservative variants of these peptides, may be employed in this invention so long as that peptide fragment demonstrates the requisite neurotoxicity.

While the peptide of SEQ ID NO:1 and SEQ ID NO:2 are referred to as β-amyloid peptide throughout this document, in the body of literature concerning this field, this peptide is alternatively referred to as β-amyloid protein, amyloid β peptide, amyloid βA4, β protein, amyloid A4, β-peptide, and other such names.

The term "treating" (or "treat") as used herein includes its generally accepted meaning which encompasses prohibiting,

preventing, restraining, and slowing, stopping, or reversing progression, severity, or a resultant symptom. As such, the methods of this invention encompass both therapeutic and prophylactic administration.

The term "effective amount" as used herein refers to the amount of compound necessary to treat physiological effects or disorders associated with β-amyloid peptide, or inhibit amyloidogenic production or deposition, or treat Alzheimer's Disease, as the case may be.

The term "physiological disorder associated with β-amyloid peptide" includes diseases related to the inappropriate or undesirable deposition of β-amyloid peptide, and as such includes Alzheimer's Disease (including familial Alzheimer's Disease), Down's Syndrome, advanced aging of the brain, hereditary cerebral hemorrhage with amyloidosis of the Dutch-type (HCHWA-D), and the like.

The compounds used in the method of the present invention may have one or more asymmetric centers. As a consequence of these chiral centers, the compounds of the present invention occur as racemates, mixtures of enantiomers and as individual enantiomers, as well as diastereomers and mixtures of diastereomers. All asymmetric forms, individual isomers and combinations thereof, are within the scope of the present invention.

The terms "R" and "S" are used herein as commonly used in organic chemistry to denote specific configuration of a chiral center. The term "R" (*rectus*) refers to that configuration of a chiral center with a clockwise relationship of group priorities (highest to second lowest) when viewed along the bond toward the lowest priority group. The term "S" (*sinister*) refers to that configuration of a chiral center with a counterclockwise relationship of group priorities (highest to second lowest) when viewed along the bond toward the lowest priority group. The priority of groups is based upon their atomic number (in order of decreasing atomic number). A partial list of priorities and a discussion of stereochemistry is contained in NOMENCLATURE OF ORGANIC COMPOUNDS: PRINCIPLES AND PRACTICE, (J.H. Fletcher, et al., eds., 1974) at pages 103-120.

In addition to the (R)-(S) system, the older D-L system may be used in this document to denote absolute configuration, especially

with reference to amino acids. In this system a Fischer projection formula is oriented so that the number 1 carbon of the main chain is at the top. The prefix "D" is used to represent the absolute configuration of the isomer in which the functional (determining) group is on the right side of the carbon atom at the chiral center and "L", that of the isomer in which it is on the left.

In order to preferentially prepare one optical isomer over its enantiomer, the skilled practitioner can proceed by one of two routes. The practitioner may first prepare the mixture of enantiomers and then separate the two enantiomers. A commonly employed method for the resolution of the racemic mixture (or mixture of enantiomers) into the individual enantiomers is to first convert the enantiomers to diastereomers by way of forming a salt with an optically active acid or base. These diastereomers can then be separated using differential solubility, fractional crystallization, chromatography, or like methods. Further details regarding resolution of enantiomeric mixtures can be found in J. Jacques, et al., ENANTIOMERS, RACEMATES, AND RESOLUTIONS, (1991).

In addition to the schemes described above, the practitioner of this invention may also choose an enantiospecific protocol for the preparation of these compounds. Such a protocol employs a synthetic reaction design which maintains the chiral center present in the starting material in a desired orientation. These reaction schemes usually produce compounds in which greater than 95 percent of the title product is the desired enantiomer.

In addition to the (R)-(S) system of stereochemistry, some of the compounds employed in the methods of the present invention also have the capacity for (E)-(Z) isomerism. In this system the group of higher priority bonded to one of the carbon atoms sharing the double bond is compared to the group of higher priority bonded to the other carbon atom sharing the double bond. If the two groups of higher priority are on the same side of the double bond, the alkene is designated (Z) (*zusammen*). If the two groups of higher priority are on opposite sides of the double bond the alkene is designated (E) (*entgegen*). As noted supra, all asymmetric forms, individual isomers and combinations thereof, are within the scope of the present invention.

This invention also encompasses methods employing the pharmaceutically acceptable salts of the compounds described herein. A compound employed in this invention can possess a sufficiently acidic, a sufficiently basic, or both functional groups, and accordingly react with any of a number of organic and inorganic bases, and inorganic and organic acids, to form a pharmaceutically acceptable salt.

The term "pharmaceutically acceptable salt" as used herein, refers to salts of the compounds of the above formula which are substantially non-toxic to living organisms. Typical pharmaceutically acceptable salts include those salts prepared by reaction of the compounds of the present invention with a pharmaceutically acceptable mineral or organic acid or an organic or inorganic base. Such salts are known as acid addition and base addition salts.

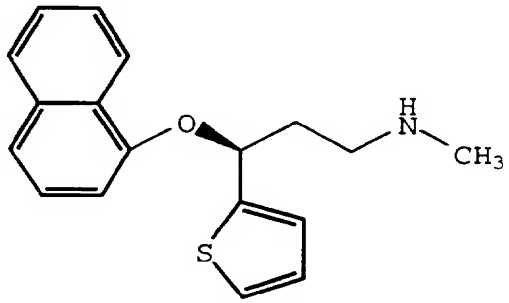
This invention also encompasses methods employing the pharmaceutically acceptable solvates of the compounds described herein. Many of these compounds can combine with solvents such as water, methanol, ethanol and acetonitrile to form pharmaceutically acceptable solvates such as the corresponding hydrate, methanolate, ethanolate and acetonitrilate.

The term "serotonin reuptake inhibitor" refers to a composition which inhibits the serotonin transporter on membranes of serotonin neurons. These uptake inhibitors increase the concentration of serotonin within the synaptic cleft by blocking its removal via the membrane transporter. Inhibitors of serotonin uptake increase serotonin action on postsynaptic receptors on target neuron, and increase serotonergic neurotransmission, resulting in functional consequences that are mostly subtle, i.e., not detectable by gross observation, but are detectable by various specific techniques.

For instance, serotonin uptake inhibitors reduce aggressive behavior, decrease food uptake, decrease alcohol drinking in rats, decrease rapid-eye-movememt sleep, potentiate morphine analgesia, and the like. R.W. Fuller, Journal of Clinical Psychiatry, 53:35-45 (1992). Serotonin uptake inhibitors are used clinically in the treatment of mental depression, bulimia, and obsessive-compulsive disorder. They are also reported to be effective as appetite suppressant drugs in the treatment of obesity, in borderline personality disorder,

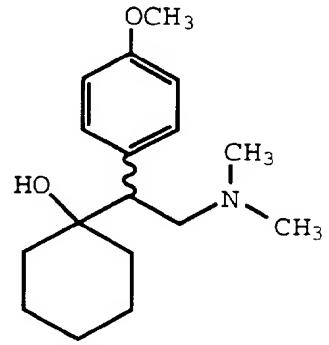
trichotillomania, panic disorder, and attention deficit hyperactivity disorder. See, e.g., R.W. Fuller, Advances in Biosciences, 85:255-270 (1992). In addition, serotonin uptake inhibitors have been reported to have therapeutic benefit in premenstrual syndrome, diabetic neuropathy, chronic pain, and in postanoxic intention myoclonus. Id.

One such compound is duloxetine which has activity as a reuptake inhibitor of both serotonin and norepinephrine. This compound has the structure



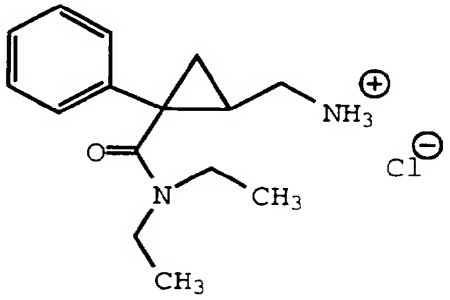
and the chemical name (S) N-methyl-3-(1-naphthalenyloxy)-3-(2-thienyl)propanamine. This compound is usually administered as the hydrochloride salt. The preparation of this compound is described in United States Patent 4,956,388, the entire contents of which is herein incorporated by reference. The term "duloxetine" as employed herein refers to any acid addition salt or the free base of the molcule.

Venlafaxine is known in the literature as a serotonin and norepinephrine reuptake inhibitor. This compound has the structure



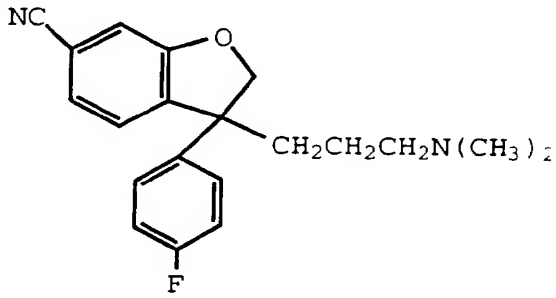
and is referred to as 1-[(2-dimethylamino)-1-4-methoxyphenyl)ethyl]cyclohexanol. This compound is taught in United States Patent 4,761,501, the entire contents of which is herein incorporated by reference.

Milnacipran {(Z) 1-phenyl-1-diethylaminocarbonyl-2-aminomethylcyclopropane hydrochloride} is taught in United States Patent 4,478,836, the entire contents of which are herein incorporated by reference. The structure of milnacipran is as follows.



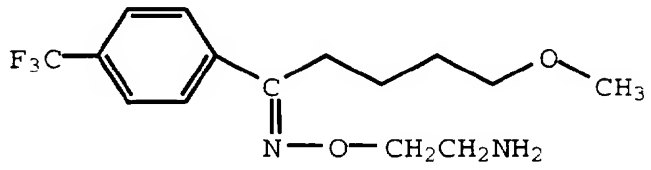
Milnacipran is known to increase the availability of serotonin, norepinephrine, and dopamine.

Another serotonin reuptake inhibitor which may be employed in the methods of the present invention is citalopram, a compound having the structure



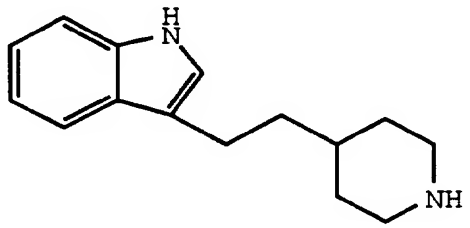
and the chemical name 1-[3-(dimethylamino)propyl]-1-4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile. This compound may be prepared as described in United States Patent 4,136,193, the entire contents of which are herein incorporated by reference.

Another serotonin reuptake inhibitor which may be employed in the methods of the present invention is fluvoxamine, a compound having the structure



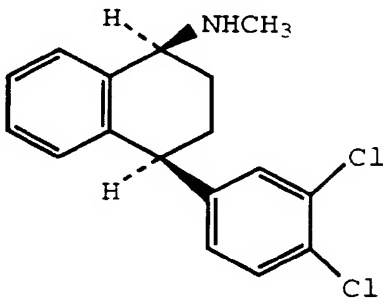
and the chemical name 5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone O-(2-aminoethyl)oxime. This compound may be prepared as described in United States Patent 4,085,225, the entire contents of which are herein incorporated by reference.

Another compound belonging to this class of therapeutics is indalpine, a compound having the structure



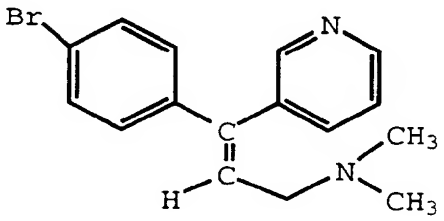
and the chemical name 3-[2-(4-piperidinyl)ethyl]-1H-indole. This compound may be prepared as described in United States Patent 4,064,255, the entire contents of which are herein incorporated by reference.

Sertraline is another serotonin reuptake inhibitor which may be employed in the methods of the present invention. This compound, having the chemical name (1S-cis)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthalenamine, has the following structure.



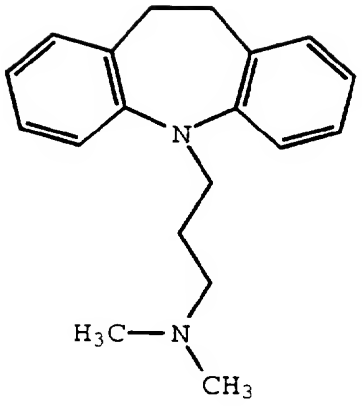
Sertraline may be prepared as described in United States Patent 4,536,518, the entire contents of which are herein incorporated by reference.

An additional such inhibitor which may be employed in the methods of the present invention is zimeldine, a compound of the structure



having the chemical name (Z)-3-(4-bromophenyl)-N,N-dimethyl-3-(3-pyridinyl)-2-propen-1-amine. This compound may be prepared as described in United States Patent 3,928,369, the entire contents of which are herein incorporated by reference.

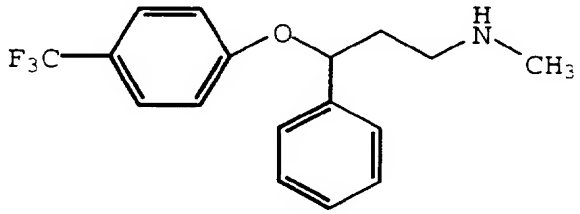
Another such compound is imipramine, a compound having the structure



and the chemical name 10,11-dihydro-N,N-dimethyl-5-dibenz[b,f]azepine-5-propanamine. This compound is prepared as described in United States Patent 2,554,736, the entire contents of which is herein incorporated by reference.

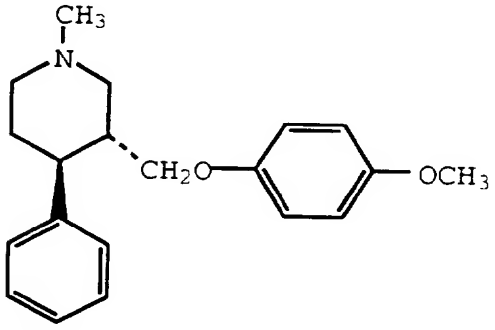
The selective serotonin reuptake inhibitors (SSRI's) are a series of compounds which act as serotonin reuptake inhibitors but act in a selective manner. Selective serotonin reuptake inhibitors are especially preferred in the methods of the present invention.

One such compound is fluoxetine, a compound having the structure



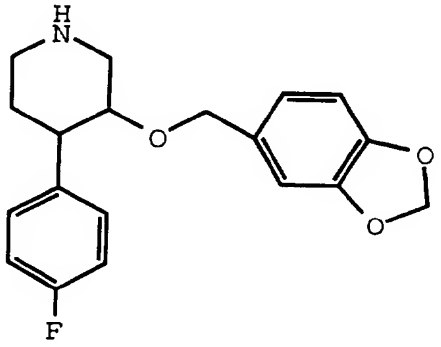
and the chemical name N-methyl-3-(4-trifluoromethylphenoxy)-3-phenylpropylamine. This compound is prepared as described in United States Patent 4,314,081 which is herein incorporated by reference. Throughout this document the term "fluoxetine" refers to any acid addition salt or the free base, and includes either the racemic mixture or either of the enantiomers.

Another compound belonging to this class of therapeutics is femoxetine, a compound having the structure



and the chemical name (3R-trans)-3-[(4-methoxyphenoxy)methyl]-1-methyl-4-phenylpiperidine. This compound may be prepared as described in United States Patent 3,912,743, the entire contents of which are herein incorporated by reference.

Another such compound is paroxetine, a compound having the structure



and the chemical name trans-(-)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine. This compound is prepared as described in United States Patents 3,912,743 and 4,007,196, the entire contents of which are herein incorporated by reference.

The above groups of compounds are only illustrative of the serotonin reuptake inhibitors which are currently under development or are frequently employed in serotonin receptor studies. This listing of groups of compounds is not meant to be comprehensive, the methods of the present invention may employ any serotonin reuptake inhibitor and is not limited to any particular class of compound.

The biological activities of the compounds of the present invention are evaluated employing an initial screening assay which rapidly and accurately measures the inhibition of β-amyloid peptide in a whole cell assay.

β-Amyloid Peptide Production Inhibition (Cellular Assay)

Two cell lines (human kidney cell line 293 and Chinese hamster ovary cell line CHO) are stably transfected with the gene for APP751 containing the double mutation Lys_{651}-Met_{652} to Asn_{651}-Leu_{652} (APP-751 numbering) commonly called the Swedish mutation using the method described in Citron, et al., Nature 360:672-674 (1992). The transfected cell lines are designated as 293 751 SWE and CHO 751 SWE, and are plated in Corning 96 well plates at $2.5x10^4$ or $1x10^4$ cells per well respectively in Dulbecco's minimal essential media (DMEM) plus 10% fetal bovine serum. Following overnight incubation at 37°C in an incubator equilibrated with 10% carbon dioxide (CO_2), the media are removed and replaced with 200 µl per well of media. After a two hour pretreatment period, the media are again removed and replaced with fresh media containing the test compound and the cells are incubated for an additional two hours.

After treatment, plates are centrifuged at 1200 rpm for five minutes at room temperature to pellet cellular debris from the conditioned media. From each well, 100 µl of conditioned media are transferred into an ELISA plate precoated with antibody 266 against β-amyloid peptide(13-28) and stored at 4°C overnight. An ELISA assay employing labelled antibody 6C6 (against β-amyloid peptide-1-16) is run the next day to measure the amount of β-amyloid peptide produced.

Cytotoxic effects of the compounds are measured by a modification of the method of Hansen, et al., Journal of Immunological Methods, 119:203-210 (1989). To the cells remaining in the tissue culture plate, is added 25 µl of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) stock solution (5 mg/ml) to a final concentration of 1 mg/ml. Cells are incubated at 37°C for one hour, and cellular activity is stopped by the addition of an equal volume of MTT

lysis buffer (20% w/v sodium dodecylsulfate in 50% DMF, pH 4.7). Complete extraction is achieved by overnight shaking at room temperature. The difference in the OD_{562nm} and the OD_{650nm} is measured in a UV_{max} microplate reader as an indicator of the cellular viability.

The results of the β-amyloid peptide ELISA are fit to a standard curve and expressed as ng/ml β-amyloid peptide. In order to normalize for cytotoxicity, these β-amyloid peptide results are divided by the MTT results and expressed as a percentage of the results from a drug-free control.

In addition to the above assay, a similar assay employing IMR32 cells possessing endogenous wild-type APP is employed in a similar manner.

In repeated experiments using the above assays, serotonin reuptake inhibitors have shown significant inhibition of β-amyloid peptide production without demonstrating increased cytotoxicity. Especially preferred are the selective serotonin reuptake inhibitors.

In another assay to better study the effects of the above-described compounds on whole animals some of the above compounds are administered in vivo to guinea pigs by an intraperitoneal route at 1 mg/kg/day. The individual compound being tested is administered in four 0.25 mg/kg doses separated by one hour. One hour after the last of the four injectons, the amount of β-amyloid peptide in the cerebrospinal fluid is measured and compared to control animals in which no such compound is administered.

While it is possible to administer a compound employed in the methods of this invention directly without any formulation, the compounds are usually administered in the form of pharmaceutical compositions comprising a pharmaceutically acceptable excipient and at least one active ingredient. These compositions can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. Many of the compounds

employed in the methods of this invention are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound. See, e.g., REMINGTON'S PHARMACEUTICAL SCIENCES, (16th ed. 1980).

In making the compositions employed in the present invention the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing for example up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxybenzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active

ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.05 to about 100 mg, more usually about 1.0 to about 30 mg, of the active ingredient. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

The active compounds are generally effective over a wide dosage range. For examples, dosages per day normally fall within the range of about 0.01 to about 30 mg/kg of body weight. In the treatment of adult humans, the range of about 0.1 to about 15 mg/kg/day, in single or divided dose, is especially preferred. However, it will be understood that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound or compounds administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, provided that such larger doses are first divided into several smaller doses for administration throughout the day.

Formulation Preparation 1

Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity (mg/capsule)
Active Ingredient(s)	30.0
Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Preparation 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity (mg/tablet)
Active Ingredient(s)	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Preparation 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient(s)	5
Lactose	95

The active mixture is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Preparation 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity (mg/tablet)
Active Ingredient(s)	30.0 mg
Starch	45.0 mg
Microcrystalline cellulose	35.0 mg
Polyvinylpyrrolidone (as 10% solution in water)	4.0 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1.0 mg
Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50-60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Preparation 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient(s)	40.0 mg
Starch	109.0 mg
Magnesium stearate	1.0 mg
Total	150.0 mg

The active ingredient, cellulose, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Preparation 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

Ingredient	Amount
Active Ingredient(s)	25 mg
Saturated fatty acid glycerides to	2,000 mg

The active ingredient(s) is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Preparation 7

Suspensions, each containing 50 mg of medicament per 5.0 ml dose are made as follows:

Ingredient	Amount
Active Ingredient(s)	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%) Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 ml

The medicament, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Preparation 8

Capsules, each containing 15 mg of medicament, are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient(s)	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

The active ingredient(s), cellulose, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425 mg quantities.

Formulation Preparation 9

An intravenous formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient(s)	250.0 mg
Isotonic saline	1000 ml

Formulation Preparation 10

A topical formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient(s)	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

Formulation Preparation 11

Sublingual or buccal tablets, each containing 10 mg of active ingredient, may be prepared as follows:

Ingredient	Quantity Per Tablet
Active Ingredient(s)	10.0 mg
Glycerol	210.5 mg
Water	143.0 mg
Sodium Citrate	4.5 mg
Polyvinyl Alcohol	26.5 mg
Polyvinylpyrrolidone	15.5 mg
Total	410.0 mg

The glycerol, water, sodium citrate, polyvinyl alcohol, and polyvinylpyrrolidone are admixed together by continuous stirring and maintaining the temperature at about 90°C. When the polymers have gone into solution, the solution is cooled to about 50-55°C and the medicament is slowly admixed. The homogenous mixture is poured into forms made of an inert material to produce a drug-containing diffusion matrix having a thickness of about 2-4 mm. This diffusion matrix is then cut to form individual tablets having the appropriate size.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, e.g., U.S. Patent 5,023,252, issued June 11, 1991, herein incorporated by

reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system, used for the transport of biological factors to specific anatomical regions of the body, is described in U.S. Patent 5,011,472, issued April 30, 1991, which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs or prodrugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

The type of formulation employed for the administration of the compounds employed in the methods of the present invention may be dictated by the particular compounds employed, the type of pharmacokinetic profile desired from the route of administration and the compound(s), and the state of the patient.

We Claim:

1. A method of treating a physiological disorder associated with β-amyloid peptide which comprises administering to a mammal in need thereof an effective amount of a composition having activity as a serotinin reuptake inhibitor.

2. A method as claimed in **Claim 1** wherein said serotonin reuptake inhibitor is a selective serotonin reuptake inhibitor.

3. A method as claimed in **Claim 2** wherein said selective serotonin reuptake inhibitor is fluoxetine.

4. A serotonin reuptake inhibitor as employed in any one of **Claims 1 to 3**, for use in treating a physiological disorder associated with β-amyloid peptide.

5. The use of a serotonin reuptake inhibitor as employed in any one of **Claims 1 to 3**, for the manufacture of a medicament for the treatment of a physiological disorder associated with β-amyloid peptide.

6. A pharmaceutical formulation adapted for the treatment of physiological disorder associated with β-amyloid peptide, comprising a serotonin reuptake inhibitor as employed in any one of **Claims 1 to 3**.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15046

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	GELDMACHER, D.S. ET AL. Fluoxetine in Dementia of the Alzheimer's Type: Prominent Adverse Effects and Failure to Improve Cognition. Journal of Clin. Psychiatry. April 1994. Vol. 55. No. 4. page 161, see entire document.	1-4 5,6
X Y	GOTTFRIES, C.G. Therapy Options in Alzheimer's Disease. British Journal of Clinical Practice. November/December 1994. Vol. 48. No. 6. pages 327-330, especially page 329.	1,2,4 3,5,6

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
Jacqueline Krikorian
Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15046

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	TOHGI, H. ET AL. Indoleamine Concentrations in Cerebrospinal Fluid from Patients with Alzheimer Type and Binswanger Type Dementias before and after Administration of Citalopram, a Synthetic Serotonin Uptake Inhibitor. Journal of Neural Transmission. 1995. Vol. 9. pages 121-131, see entire document.	1,2,4 3,5,6
X	US 5,372,813A (MATHIS ET AL.) 13 December 1994, see entire document.	1-6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15046

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/13, 31/015, 31/02; C07C 211/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/579; 564/305

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

514/888

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

CAPlus, Registry, Medline, WPIDS, EMBASE, APS
Search terms: serotonin reuptake inhibitor, fluoxetine, Alzheimer, beta-amyloid